Synthetic media have not in the past been found satisfactory for this lysogenic system[2,3]. However, the following medium C/G[4] (Table I) has been used for daily transfer of *B. megaterium* 899(1) and lysogeny has not been lost after 80 subcultures.

On the other hand, induction has never been brought about in C/G even with the further addition of trace elements and calcium. Transfer to a Bacto tryptone-Bacto yeast extract medium (TYE) immediately rendered the culture "apt" for induction. An equally effective but fully synthetic induction medium was found to be the basal salts solution, C, plus 18 amino-acids (50–400 μg/ml of each) and with or without 0.5% glucose (C/18AA or C/18AA/G). Omission of cysteine which might itself act as an inducing agent, did not impair the medium (C/17AA/G).

Figs. 1A, B and C show typical results. Various media were inoculated with a culture which had been transferred more than 20 times in C/G and when growth was vigorous, hydrogen peroxide (final concentration $M/15{,}000$) was added as inducing agent. After lysis had occurred, the phage titres of induced cultures were $5 \cdot 10^9$–$5 \cdot 10^{10}$/ml in both synthetic and complex media.

It is apparent that the property of lysogeny can be maintained for long periods of cultivation in a simple glucose/salts medium. It is also evident that induction can be achieved with the same dosage of inducing agent in glucose/amino-acids/salts medium as in complex media (contrast LWOFF[3]). Neither calcium ions nor trace elements improved the performance. The only divalent cation in the medium was magnesium at a concentration of 10 mg/litre.

KENNETH MCQUILLEN
M. R. J. SALTON

Department of Biochemistry, University of Cambridge (England)

[1] M. R. J. SALTON AND K. MCQUILLEN, in preparation.
[2] N. A. CLARKE, *J. Bacteriol.*, 63 (1952)187.
[3] A. LWOFF, *Bacteriol. Revs.*, 17 (1953) 269.
[4] K. MCQUILLEN AND R. B. ROBERTS, *J. Biol. Chem.*, 207 (1954) 81.

Received January 27th, 1955

C-terminal amino-acid sequence of tobacco mosaic virus protein

When tobacco mosaic virus (TMV) is treated with carboxypeptidase, about 2,900 residues of threonine, and only threonine, are released per mole[1,2]. This remarkable finding is in accord with other observations[3] which suggest that each virus particle contains approximately 2,900 peptide-chain sub-units. However, the release of many threonine residues occurring in sequence and other interpretations are by no means excluded. A chemical attack on the protein would give definitive data on this point and might yield further information concerning the terminal sequence.

When the protein prepared from TMV was subjected to hydrazinolysis, according to AKABORI AND OHNO[4,5], and the DNP-derivative of the free C-terminal amino acid identified and determined by 2-dimensional chromatography[6], only threonine was found. The amount of threonine released from the protein, corrected for the losses inherent in the method, corresponded to 1 equivalent per 18,000 g (Table I). Thus, the C-terminal position of about 2,800 threonine residues and only threonine, was definitely established[7].

TABLE I

RELEASE OF C-TERMINAL REACTION PRODUCTS OF HYDRAZINOLYSIS

Hydrazinolysis time	*TMV protein threonine*	*Dethreoninated TMV protein*	
		Alanine	*Prolyl-alanine*
(h)	equivalents per 18,000 g protein*		
4	—	0.09	0.48
5	0.79	0.35	0.32
10	1.00	0.94	0.08
22	0.95	0.92	trace

* Corrected by factors of loss incurred during hydrazinolysis (10 h period), dinitrophenylation and extraction of 0.62, 0.63 and 0.63 for threonine, alanine and prolyl-alanine respectively. Most data are the averaged results of several runs, usually with about 0.5–1.0 μM of protein.

When the protein isolated from enzymatically dethreoninated virus was reacted with hydrazine for 10 or 22 hours, only one free amino acid, isolated as the DNP-derivative, was found in near-stoichiometric amount. This amino acid was identified as alanine. In addition, a trace of an unknown

DNP-derivative was detected on these chromatograms. Shorter reaction periods increased the latter, and yielded less alanine. The highest yield of the unknown material was obtained after 4 h of hydrazinolysis (see Table I). The U.V. absorption spectrum suggested that this material was related to, but not identical with, proline. The observed maximum of 375 mμ would be characteristic for a DNP-prolyl-peptide[8]; the disappearance of the material during longer hydrazinolysis is in accord with such an hypothesis. The material, after elution from a chromatogram, was therefore subjected to acid hydrolysis (5.7 *N* HCl, 5 h reflux). Dinitrophenol and traces of both DNP-proline and the unhydrolyzed DNP-peptide were found in the ether extract. In contrast, chromatographic analysis of the aqueous phase indicated the presence of equimolecular amounts of two free amino acids, proline and alanine; these were identified by their R_F values (Butanol-acetic acid: 0.35, 0.33; standards 0.35, 0.33. Buffered phenol-cresol: 0.80, 0.33; standards 0.80, 0.33), and differentiated by the isatin and ninhydrin test. It thus appears, that prolyl-alanine is split rapidly from the C-terminal position of the dethreoninated virus protein and can be isolated as the DNP-derivative. Its spectral characteristics and its lability in acid and hydrazine are in full accord with this interpretation[8]. Longer hydrazinolysis decomposes this peptide and yields only the C-terminal alanine in the acid fraction.

Thus, the C-terminal sequence in the TMV protein is -prolyl-alanyl-threonine.

CHING-I. NIU*
H. FRAENKEL-CONRAT**

Virus Laboratory, University of California, Berkeley, Calif. (U.S.A.)

[1] J. I. HARRIS AND C. A. KNIGHT, *Nature*, 170 (1952) 613.
[2] J. I. HARRIS AND C. A. KNIGHT, *J. Biol. Chem.*, (in press).
[3] C. A. KNIGHT, *Advances in Virus Research*, Vol. II, (1954) p. 153.
[4] S. AKABORI, K. OHNO AND K. NARITA, *Bull. Chem. Soc. of Japan*, 25 (1952) 214.
[5] K. OHNO, *J. Biochem. (Japan)*, 41 (1954) 345.
[6] A. LEVY, *Nature*, 174 (1954) 126.
[7] During the preparation of this manuscript, a paper by G. BRAUNITZER (*Z. Naturforsch.*, 9b (1954) 675) came to our attention, which also demonstrates the C-terminal position of threonine in TMV protein by hydrazinolysis. Other conclusions contained in that paper appear not valid. No N-terminal groups are found in repeated reinvestigations of this problem in this laboratory (see H. FRAENKEL-CONRAT AND B. SINGER, *J. Am. Chem. Soc.*, 76 (1954) 180).
[8] H. FRAENKEL-CONRAT, J. I. HARRIS AND A. L. LEVY, in *Methods of Biochemical Analysis*, Vol. II, Ed. . . GLICK, Interscience Publ., N.Y., 1955.

Received February 3rd, 1955

* Aided by a grant from the Rockefeller Foundation.
** Aided by a grant from the National Foundation for Infantile Paralysis.

Répartition de la radioactivité sur le D-ribose biosynthétique

Dans une publication antérieure[1], nous avons montré que le ribose de l'acide nucléique de *Torula utilis* cultivée sur D-glucose·2^{14}·C comme unique source de carbone possède une radioactivité par molécule très voisine de celle du glucose. On en retrouve environ 93% sur un fragment qui groupe les carbones 1, 2, 3. Le carbone 5 est inactif. Nos nouvelles expériences montrent que l'activité est entièrement portée par le carbone aldéhydique du ribose.

Réduction du D-*ribose en* D-*ribitol*

A une solution de D-ribose (250 mg) dans l'eau (5 ml) on ajoute une solution de borohydrure de sodium (35 mg) dans l'eau (2.5 ml). On laisse une nuit à température ambiante, puis on décompose l'excès de réactif par addition d'acide acétique jusqu'à neutralité, on déionise sur une colonne mixte Duolite-Zeo·Karb et on évapore à sec sous vide à 30–40°. Par cristallisation dans l'alcool on obtient le D-ribitol (186 mg — 75% F. 97–98°, s'élevant à 99–101° après une recristallisation). On n'a pas observé la formation parasite d'esters boriques.

Isolement du D-*ribose à l'état de* D-*ribitol*

Nous avons opéré exactement de la façon décrite[1] pour la culture de *Torula utilis*, avec les mêmes poids de composés, mais après hydrolyse de l'acide nucléique et déionisation, au lieu de séparer le ribose biosynthétique à l'état d'osazone, nous avons d'abord vérifié par chromatographie sur papier que le résidu ne contenait pas d'autre sucre que le ribose.

On ajoute alors du D-ribose inactif comme entraîneur (20.0 mg) et l'ensemble, dissous dans l'eau (0.5 ml) est réduit par le borohydrure de sodium (10 mg) dans l'eau (1 ml). On procède comme